SOME EXPERIMENTS ON STERILISATION BY STEAM.

It is generally admitted that, whenever it is applicable, disinfection by steam is the best process of disinfection which we possess. But there is not the same unanimity of opinion with regard to the best method of using steam for that purpose. Some observers are of opinion that current steam at the ordinary atmospherical pressure, or at a pressure very slightly above that, is capable of killing all pathogenic germs within a few minutes. Many authorities on the other hand say that, in order to ensure the death of many sporing organisms within a time suitable for practical operations, it is necessary to use either superheated steam or steam under pressure.

It is not my intention to discuss here the physical properties of steam under these various conditions, and therefore I will pass over the observations and theoretical considerations which prove clearly that superheated steam, instead of having any advantage over saturated steam, has few of the advantages of steam and many of the disadvantages of hot air for disinfecting purposes. These disadvantages are, however, obscured in practice by the fact that superheated steam becomes rapidly saturated steam when it comes in contact with cold objects.

Saturated steam has very much the same properties whether it be under low or high pressures, with that difference that steam generated at the ordinary atmospherical pressure has a temperature of 100° C., whilst steam generated under higher pressures has higher temperatures, as shown by the following table (Dulong and Arago):—

1·0 atmosphere	100° C.
1·5 ,,	112·2°
2·0 atmospheres	121·4°
2·5 ,,	128·8°
3·0 ,,	135·1°
3·5 ,,	140·6°
4·0 ,,	145·4° &c.

Steam is seldom used for disinfecting purposes at a pressure of more than 20 to 22 pounds to the square inch; that is to say, it is generally used at a pressure of much less than that of two atmospheres. Such a pressure has practically no effect on the vitality of bacteria. It is therefore not on account of the pressure, but on account of the increased temperature that high-pressure is preferred, by many, to low-pressure steam. But is it necessary to raise the temperature of a microbe to more than 100° C. in order to kill it? Simple physiological considerations would seem to point out that long before that temperature has been reached all the proteid substances which form the substrata of life would have been coagulated and incapable of life. The heat-coagulation temperature of one variety of serum albumen is the only one which reaches 84° C. Other proteid substances are coagulated at a lower—and often much lower—temperature.

The question is therefore not so much to find the means of raising the temperature of the steam to a point much above that of boiling water, but to find the means of heating the bacteria up to a temperature and under conditions ensuring the rapid coagulation of their proteid constituents.

If bacteria were large bodies, the conduction of heat from their surface to their centre would take a considerable time, and there might be some reason to believe that an increase of temperature of the disinfecting medium would be very advantageous. But as the largest microbe is an exceedingly small body, it is probable that if any of its parts were heated by a constant source of heat somewhat above that of the temperature of coagulation, the whole of that microbe would in a very short time be sufficiently heated to make its death certain.

In order that this result be obtained, however, nothing should intervene between the protoplasma of the microbe and the source of heat, for if a still layer of a non-conducting substance, such as air, separated the microbe from the source of heat, such a layer would for a long time prevent the transmission of heat to the central parts of the microbe.

It is probable that the spores of many microbes have, especially after they have been dried, the power to retain, either on their surface or in their envelope, a certain amount of air or gas, and that in this way they are protected for a considerable time from the action of heat. Tyndall's well-known experiments seem to me to be better explained in that way than on the hypothesis which he advanced at the time. He found that vegetable infusions (probably containing the spores of many earth bacilli) could be boiled for several hours without being sterilised when free access of air was permitted. In the absence of air, on the contrary, complete sterilisation could generally be obtained in a few minutes.

To obtain data admitting of strict comparison on this point I have devised a number of experiments, which cannot be recorded in detail here, but the main results of which are quite sufficient for the present purpose.

They will be published in detail before long. Spores of the bacillus anthracis were exposed to the action of constant heat, = 100° C., in various ways.

(1) After having been dried over sulphuric acid they were enclosed in tubes full of air also dried over sulphuric acid.

(2) Having been dried in the open air, they were enclosed in tubes containing air not dried over sulphuric acid.

(3) Having been dried in the usual way, or over sulphuric acid, they were exposed to the action of a mixture of air and steam.

(4) Having been dried in the usual way, or over sulphuric acid, they were exposed to the action of current steam free from air.

The results were as follows :—Some of the spores kept in dry air resisted the action of a temperature of 100° C. for 8 hours. (If precautions had been taken to prevent the contact of the spores with the walls of the tube containing them it is probable that they would have resisted longer still.) The spores which were not in perfectly dry air were killed in less than 5 hours. The spores which were exposed to the action of a mixture of steam and air were killed in $\frac{1}{2}$ hour. The spores exposed to the action of steam in the absence of air were killed in from 30 to 35 seconds. In these experiments all the conditions were identical, with the exception of those relating to the presence of air and water vapour. It is therefore evident that the differences observed are due to these two factors, or to one of them.

A temperature of 100° acting on spores, dried to such a degree that life is still possible, is capable of killing them in a certain number of hours.

A slight amount of moisture in the air and in the spores diminishes the length of time necessary to bring about the death of these spores. This may be explained partly by saying that coagulation takes place more readily when a certain proportion of water is present, and partly by supposing that the admixture of a small quantity of water vapour helps the transmission of heat to the spores. The latter view is, however, very problematic, because the small amount of water vapour present in the air would not be enough to bring that vapour to the point of saturation, and under these conditions the vapour would not act better than air for the transmission of heat.

As soon, however, as the proportion of water is sufficient to bring the steam generated to the point of saturation, then the time necessary to obtain sterilisation is reduced from hours to minutes. Here the action of steam becomes evident. The heating of the spores is comparatively so rapid that we must admit that it takes place by some process different from convection ; that is to say, the water which has been vaporised must, by condensing on the spores, give up to them the latent heat of vaporisation, by which it was kept in the gaseous state. Yet the spores are apparently still protected by the air present round them.

When, on the contrary, no air is left in the apparatus, and a sharp current of saturated steam at 100° is made to pass over them, they are killed in about the 1,000th part of the time which would be necessary to obtain the same result with dry air heated exactly to the same extent. As, in my experiments, the rapid passage of steam over the spores was the only means used to remove the air, it is evident that the rapid action of current steam must be attributed partly to the displacement of air produced by it and partly to the setting free of the latent heat of vaporisation.

Now if the presence of air is such an important factor in disinfection, all steam-disinfecting machines should be constructed in such a way that air can be expelled from them as rapidly as possible when thorough disinfection is wanted.

This has been realised lately by many makers of steam disinfectors. Those who believe in the use of steam under pressure recommend intermittent relaxation of the pressure by allowing steam to escape at intervals freely from the sterilising chamber, which is allowed to refill between these relaxations with steam at the pressure they want to obtain. Others have added to their machines contrivances for removing more or less completely the air they contain, and producing a more or less complete vacuum in the disinfecting chamber.

But I am convinced that if the action of steam at low pressure had been clearly understood, such complications would have been found unnecessary.

First of all, in order to obtain steam under pressure it is necessary to confine it by opposing to its escape some valve blowing off only when the pressure wanted has been reached.

Now this confinement of steam interferes with the free escape of air. It is true that the valves may be brought into action only when steam begins to escape freely from the apparatus, that is to say, the disinfector may be used at first as if it were intended for current steam at low pressure, and afterwards, the outlet being closed, the steam may be allowed to reach the pressure wanted. Unfortunately for this plan, most disinfectors intended to work under pressure are provided with comparatively small steam outlets as well as inlets, and when they contain bulky, porous articles, such as mattresses, it is not easy to judge from the appearance of the steam jet whether the air contained in the chamber has escaped entirely or not. On the other hand, it would appear, from the experiments I have yet to relate, that if steam is allowed to escape freely, until all the air is driven out of the chamber, it is practically certain that the disinfection is already complete, or nearly so, before the pressure is put on. If I am right in saying this, the question will be asked, Why is it necessary to construct, at a considerable cost, disinfectors capable of withstanding a high pressure, since they do their work best when used at low pressure ? The answer is simply that many observers, having obtained excellent results with high-pressure machines, have not sufficiently considered whether the same results could not be obtained with low-pressure disinfectors. That I am not alone in holding that view is shown by the fact that several forms of low-pressure disinfectors are in use abroad and in this country. Among these may be mentioned the Schimmel's, one of the Geneste Herscher's, the Thursfield's, the Reck's, and the Thresh's disinfectors.

In 1889 I made some experiments on this point which led me to adopt for laboratory purposes a very simple form of steam disinfector, which gave me results as good as those obtained with the much more expensive but otherwise excellent autoclave designed by Chamberland, and which acted much more rapidly than the Koch steam steriliser.

I was led to construct this apparatus partly for purposes of economy and partly because I had been struck with the extraordinary efficiency of current steam when the current was bulky and rapid.

This apparatus, which I described in 1891,* was constructed as shown on page 6 :—

(*a*) A deep, flat-bottomed dish about 35 c.m. in diameter, held at a proper height over a powerful Bunsen burner.

(*b*) A metallic bell jar 25 to 30 c.m. in diameter and 40 c.m. high, with a hemispherical or conical top provided with a central large tubulure, into which a cork is fitted. In the cork are openings for the escape of steam and, if necessary, the insertion of thermometer and thermo-regulator. For purposes of experimental observation this metallic bell jar was replaced by a glass bell of the same size.

(*c*) A shallow, flat-bottomed dish of diameter slightly less than that of the jar, with lateral perforations close to its bottom, and placed on an inverted position in the centre of the first dish. This last part of the apparatus is not absolutely necessary, except as a support and to prevent the splashing resulting from the violent ebullition of the water.

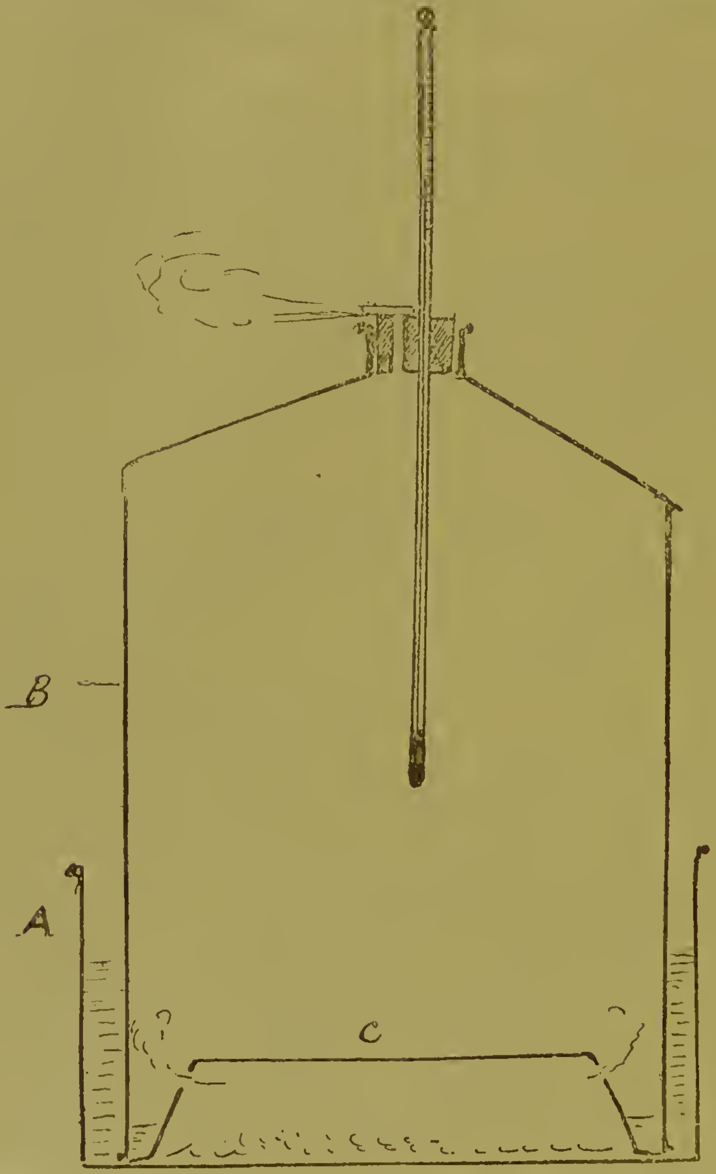
Three hundred to 400 c.m. of water being poured into the large dish and the bell jar being placed over it, the whole apparatus is now composed of a chamber, the bottom of which is formed by the large dish containing the water, and the sides and roof of which are provided by the bell jar, the only outlets for any steam produced being the upper opening, and, when the pressure within the jar is sufficient to press all the water out of it, the narrow fissure left between the lower border of the bell jar and the bottom of the large dish on which it stands and against which it is pressed only by its own weight.

---

* Transactions of the Seventh International Congress of Hygiene and Demography, London. Vol. II., p. 328.

Such an arrangement allows one to study carefully the effects of the generation of steam. For this purpose we may put aside the third part (*c*) of the apparatus.

The upper opening is closed by means of a cork, through which a thermometer is introduced, so that the bulb indicates the temperature of the upper half or middle of the chamber. Another opening 6 m.m. in diameter allows the steam and air to escape from the chamber. The size of this opening can be reduced by the insertion of glass tubes narrowed to various degrees, or it may be covered with small flat pieces of glass or metal of various weights, so as to obtain a slight increase of pressure within the apparatus.



The first thing that becomes evident after heating the water is that it is not enough to bring the water to the boiling point to keep the chamber filled with steam. If a small flame, capable of keeping the water boiling continuously, is used, steam will escape through the outlet intermittently, and from time to time, instead of steam escaping through the outlet, air will rush in. This is the result of an intermittent cooling of the walls of the chamber, which takes place whenever the slightest draught blows over the apparatus. This will take place whether the heating flame is steady or not, but becomes very much more marked when the flame is unsteady, as in such a case the rate at which the steam is generated is variable. The effects of the condensation of steam are well shown by the thermometer, which exhibits constant oscillation of 1° to 3° C.

A much more steady temperature and escape of steam are obtained when the water, instead of being made to boil slowly, is made to boil briskly by using a powerful source of heat. Under the first condition it is difficult or impossible to purge the chamber from air; under the second all the air can be driven out in a few minutes, as can be proved by one of the methods which will be described further on.

The second fact of importance which I wish to point out is that when steam is generated under the conditions which I have described it is impossible to obtain a temperature of 100° C. in the chamber, even when steam is escaping freely and abundantly through the outlet. The temperature is often 2°, 3°, and even 5° C. below that of the boiling point. Putting aside the effects of the low altitude at which my experiments were performed, which accounts only for a small fraction of this difference, it is evident that the loss of temperature is mostly due to the condensation taking place on and near the walls of the steam chamber.

To compensate for these losses it is necessary to cause the steam as it escapes to overcome a pressure of about 2 to 3 lbs. to the square inch in order to raise the temperature of the boiling point of water, by which means the walls of the steam chamber are heated to a higher degree than previously. The loss due to condensation thus brings back the temperature of the chamber to 100° or 99·5° C. With a non-jacketed chamber of the form described it is difficult to obtain a constant temperature of 100° without using a very powerful source of heat. The effects of intermittent cooling are manifest even in a room the air of which is comparatively still.

In the experiments in which the opening of outlet is closed by any valve, even the lightest, the effects of that cooling can be well seen by watching the thermometer and the water in the dish supporting the steam bell.

Whilst the water is boiling briskly and the steam escaping freely from under the flap acting as a valve the temperature registered is usually 99·5° C., and the water is forced from inside the bell into the annular space left between the bell and the sides of the dish.

Suddenly the steam ceases to escape from the outlet, the temperature falls rapidly by ½°, 1°, and even 2°, and the water, equally rapidly, passes back from the annular space into the bell, from which it is expelled less rapidly when the boiling water has thrown a sufficient amount of steam to fill again the chamber, as shown by the jet escaping at the top and the rise of temperature as indicated by the thermometer.

Now the movements of the water indicate how air would have rushed into the steam chamber had the valve not prevented its entrance.

These observations show the points which have to be attended to in the construction of low-pressure disinfecting machines.

(1) Steam must be generated rapidly and in large quantities to displace the air and to replace rapidly any steam that may have been condensed. This condensation otherwise would produce a partial vacuum in the steam chamber and cause an inrush of air.

(2) The walls of the steam chamber must be protected from external cooling influences, or kept at a constant temperature (not necessarily but preferably at least that of boiling water) in order to prevent condensation, or to reduce it to a constant quantity.

(3) The steam outlet must be large enough to allow a free escape of steam, but it must be guarded by a valve preventing any sudden inrush of air.

Notwithstanding all the defects which the small experimental apparatus which I have just described presented, I found it an exceedingly reliable and rapid disinfector whenever the heat applied was sufficient to keep a good current of steam passing through the apparatus.

None of the bacteria or moulds, sporing or non-sporing, usually employed in disinfection experiments lived after being left in this disinfector for intervals varying between a few minutes and one hour. As I have no complete record of these experiments made seven years ago I will not describe them at greater length. This is all the more unnecessary, as I have a recent series of similar experiments made with a much larger apparatus and giving even better results.

The chief interest of these early experiments is that, although the main temperature of the disinfecting chamber was seldom above 99° C., and often below 96°, the bactericidal action of steam was invariably perfect when care had been taken to expel all the air from the chamber.

This led me to investigate the question of the escape of air from vessels in which water was boiled, or into which steam was admitted.

In my first experiments made before 1894 I used an extremely simple method. I conducted by a pipe the air and steam escaping from a vessel in which water was heated into a basin containing recently boiled water (to ensure the absence of air as far as possible). I collected the air escaping from the tube in a glass jar of suitable size, and filled also with boiled water. I then noticed at what rate the air escaped from the boiler as the temperature in it increased. Without entering into many interesting details, it will be enough to state that the most rapid escape of air takes place when the temperature of the water is approaching the boiling point, and that, provided the size of the outlet tube is not very small, the air is practically expelled from the boiler in less than a minute after it has become filled with steam at 100° C.

At the end of 1894, Dr. Charles Porter, of Stockport, having pointed out to me the difficulty of finding out when the air had been completely expelled from the disinfecting chambers of large disinfectors employed for public purposes, I modified this method by estimating the amount of air present in samples of steam taken from time to time from the disinfector whilst disinfection was going on.

This can be done very simply by allowing steam to escape by a small pipe entering a small reservoir, from which the steam can escape by another tube. The small reservoir can be disconnected from the external air and from the disinfecting chamber by two stop-cocks, which can be opened and closed simultaneously. If, after allowing the steam or mixture of air and steam to pass freely through the reservoir, this reservoir is disconnected and cooled, a more or less complete vacuum is produced, according as there is little or much air in this reservoir.

The amount of air can be easily estimated by trapping it in a graduated gauge. The results obtained with this gauge, applied to an experimental disinfector measuring 2 feet in height by 21 inches in diameter, were practically identical with those obtained in experiments made on a smaller scale.

By causing the tube connected with the

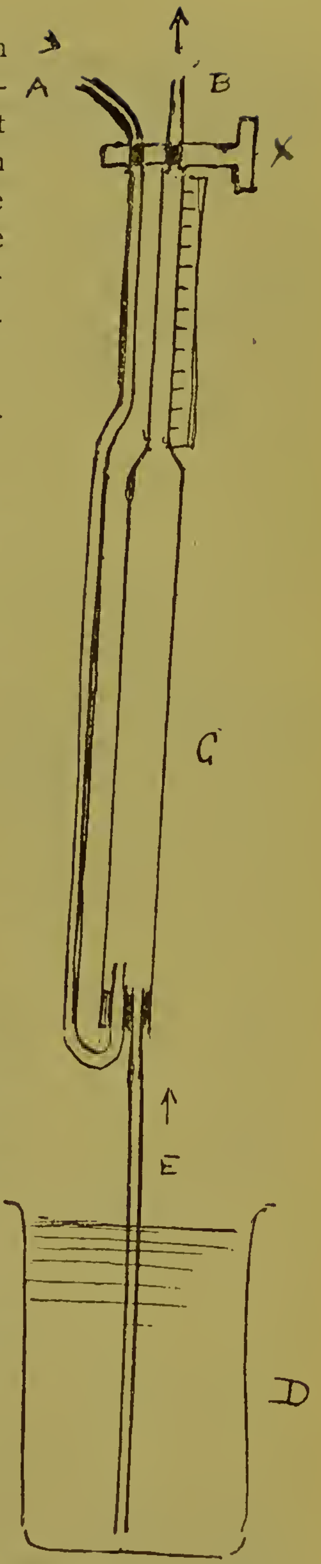
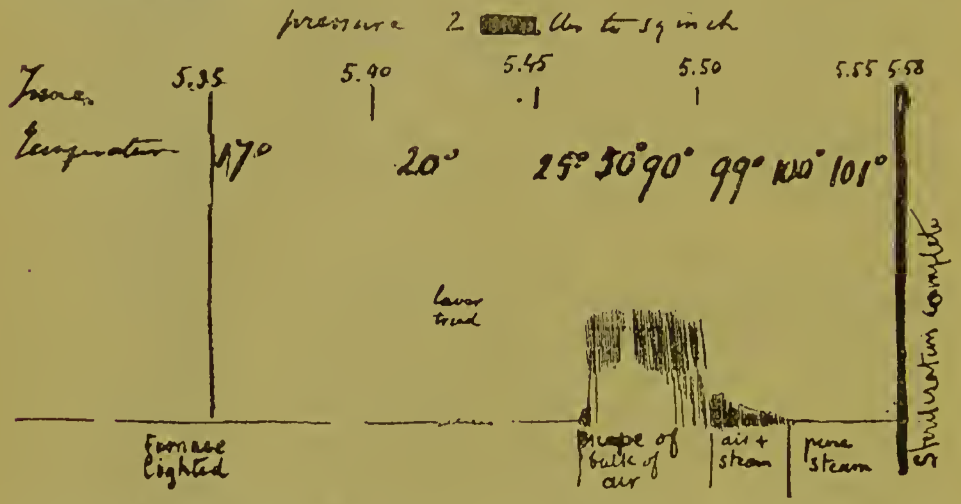
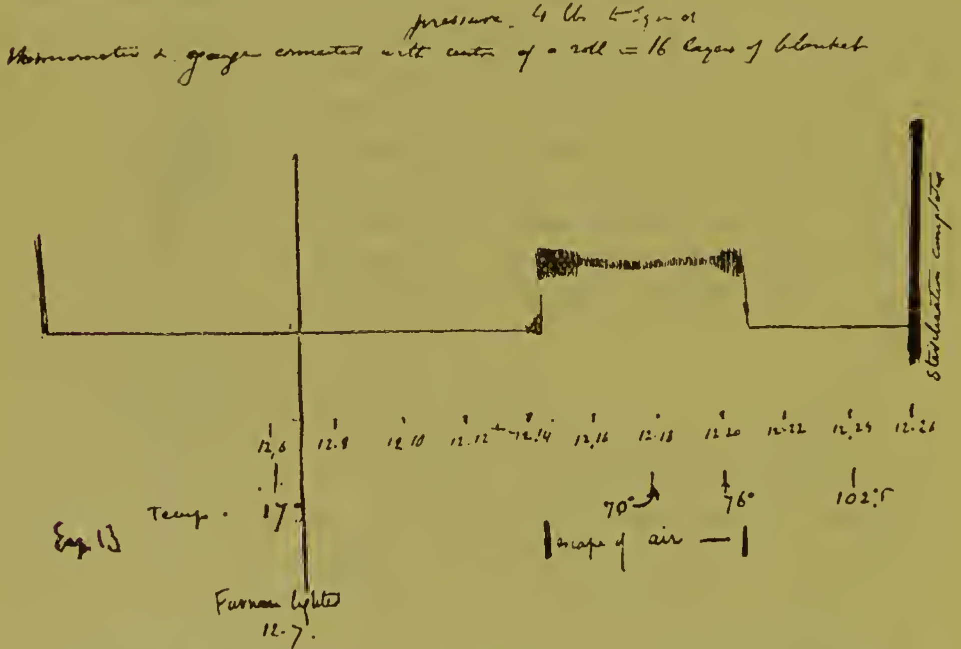


Diagram of the Steam-air Gauge.—A, pipe from boiler or steam chamber. B, outlet opening. C, gauge constricted in upper part to measure small quantities of air. D, vessel full of boiled water. E, tube through which water is driven from C when steam is admitted and passes through the gauge, and ~~as pivoted~~ aspirated from D when steam condenses after stop-cock X has been closed.

gauge to open internally in various parts of the steam chamber I was able to satisfy myself that air was displaced from all parts of the chamber practically at the same rate.



Curve showing the escape of air from the steam chamber (20 inches by 24 inches). A rise of the lever indicates an escape of air. By weighting the lever, air escaping slowly and under slight pressure is prevented from having any effect on the lever, so that the curve indicates only the rapid escape of air which takes place as soon as the water begins to boil. That is some time before the temperature of the steam chamber has reached 100° C.



Curve showing the escape of air from the centre of a blanket rolled so as to interpose sixteen layers between the cavity of the steaming chamber and the inner opening of the tube leading to the recording apparatus.

Further, by surrounding the opening of the gauge tube within the steam chamber with sixteen layers of ordinary woollen blanket so securely fastened as to make it impossible for steam to escape through that tube without passing through the sixteen layers, I found that the air was displaced exactly as in the rest of the boiler, only more slowly, the same relation existing between the displacement of air and the temperature as when the thermometer and the internal opening of the gauge tube were exposed freely in the steam chamber.

By means of a simple automatic arrangement I have recorded by a graphic method the escape of air. The curve so obtained gives the means of ascertaining when the escape of air ceases and that of pure steam begins. Such a curve will make clear better than any description the various facts to which I have just alluded.

All these experiments were made with a low-pressure steam steriliser, constructed specially for the purpose of testing the value of current steam for disinfecting purposes.

This steriliser was constructed on the same principle as the simple one described above, but the details of construction were different.

It was built about three years ago to meet the requirements of Dr. Niven, of Manchester, who was anxious to have a very portable disinfector, suitable for the disinfection of the clothes of the inmates of houses where cases of infectious illness had occurred.

The size of the disinfecting chamber being 21 inches by 20 inches, about 9 or 10 litres of water had to be heated to fill this chamber with steam without any chance of the water running short. To heat rapidly that quantity of water I used the "intensive oil furnace" (B. A. Hjorth & Co., patent), made in Stockholm. In this furnace petroleum is volatilised by heat, and escapes under pressure through a burner ensuring thorough admixture with air. With a furnace provided with six such burners, it is easy to raise the above quantity of water to the boiling point in less than 10 minutes, and to fill the whole apparatus with steam at 100° in less than 15 minutes. By using a smaller amount of water quite compatible with safety, *i.e.*, 7 litres (this corresponds to a layer of water a little more than 1 inch in thickness), the whole apparatus may be raised to a temperature of 100° C. in 8 minutes. I tried various other forms of heating arrangements, but none gave me results equal to those obtained with the intensive furnace.

In addition to these arrangements the disinfecting chamber is provided with a narrow jacket, opened both at the top and at the bottom, into which the boiling water is forced when the pressure is sufficiently high in the boiler. The boiler itself is separated from the steam chamber by a diaphragm, which allows the steam to pass freely, but retains any water which might be projected from the surface of the water when boiling violently.*

---

* The total height of the cylinder, including boiler and steam chamber, is 24 inches, and the diameter, including the jacket, is 22 inches.

The openings in the diaphragm are so arranged that they distribute the steam all round the basis of the steam chamber, and oppose just enough resistance to the passage of steam, from the boiler into the steam chamber, to cause the pressure in the former to be a little higher than that on the latter, this ensuring the forcing of hot water into the jacket.

Finally the steam chamber is closed by a lid which can be clamped firmly, provided with a large opening guarded by a dead-weight valve, opposing a resistance to the escape of steam equal to 2—4 pounds to the square inch. This valve is not essential; by reducing the size of the outlet the small amount of pressure necessary might be obtained without it; but the existence of a large outlet adds to the safety of the apparatus, and by turning off the valve the pressure within the chamber can be relieved in an instant, and the lid removed without any danger, even whilst the apparatus is full of steam at 100° C. The valve also prevents the possibility of any inrush of air. These details being known, there remains only to relate the results of numerous experiments made to find out whether, when applied in practice on a sufficiently large scale, the principles which I have explained above were supported by the results. I may say at once that they were.

As I have no exceptions to record, I can summarise the results obtained in the following way:—

In all cases it was found that steam at 100° could be obtained in from 7 minutes (minimum) to 25 minutes (maximum), the differences being due to the quantity of water, which in the quickest experiments was 7 litres, and in the slowest 15 to 20 litres. The intensity of the furnace was also an important factor. Five minutes after the temperature of 100° C. had been reached none of the microbes placed in the steriliser exposed freely or protected from the access of steam in various ways were found alive.

The bacteria thus exposed were the following:—

Staphylococcus pyogenes aureus ...	Potato cultures, agar cultures, dried films.
Bacillus typhi abdominalis	Agar cultures, recently dried films.
Bacillus coli communis	Agar cultures, recently dried films.
Bacillus prodigiosus	Potato cultures, dried films.
Bacillus tuberculosis in sputum ...	Large drops, dry and recent.
Bacillus anthracis, non-sporing or with only young spores.	Agar and bouillon cultures.
Bacillus anthracis spores, dried on paper and on silk threads.	Agar cultures.
Penicillum glaucum	Potato cultures dry.
Aspergillus flavus	Dry potato cultures. Silk threads and porous paper impregnated with conidia.
Aspergillus fumigatus	
Aspergillus niger	
Mucor corymbifer	Dry potato cultures.

The material used for experiment was invariably tested by control cultivations made in every respect under the same conditions as the disinfection experiments, with the exception, of course, of the stay in the steriliser.

The cultures in bouillon or on agar were left in the tubes in which they had grown, the tubes being plugged with cotton wool in the usual way.

The cultures on potato were either left in tubes as above or in the glass capsule containing the slice of potato on which the organism had grown.

The strips of filter paper, or the silk threads impregnated with dense emulsions of the various organisms used, were placed in narrow tubes $\frac{1}{4}$ inch in diameter and 3 or 4 inches long, constricted at their mouth so as to leave only a very narrow opening, less than $\frac{1}{16}$ inch, through which air might escape or steam penetrate. Some of these tubes were held by means of a thread passing through an opening in the lid of the steriliser, and removed half a minute, one minute, and five minutes after the temperature had reached 100° C. Dry anthrax spores exposed in this way were invariably killed.

The cultures on agar and in bouillon were placed in beakers without further protection.

The cultures on potato were exposed in different ways. Some were simply laid unprotected on the chamber. Some were wrapped up in paper and placed in the various pockets of a coat, which was afterwards rolled up and tied with string so as to make of it a parcel as compact as possible. Some were placed in a blanket folded so as to secure a minimum of eight layers in some experiments, and of sixteen layers in other experiments. (The number of layers necessarily was respectively sixteen or thirty-two on one side of such bundles, for, in order to secure perfect closure of the bundles, the ends were turned over and tied tightly with string at two or three places.) The tubes containing porous paper or silk threads impregnated with bacteria or moulds were wrapped up in three or four layers of paper twisted at each end of the tube, and were placed either free in the steriliser, in coat pockets, or within eight or sixteen folds of blanket.

Every one of the organisms mentioned was submitted to all these tests.

All the tubes inoculated with microbes exposed to the sterilising process were watched for at least one week, and generally three or four weeks, to discover any evidence of delayed growth.

Thus it was possible, with the simple disinfector I have described, in which nothing but current steam at low pressure was used, to obtain complete disinfection of articles contaminated with micro-organisms of well-known resistance, and protected in a way which made it necessary for the steam to have strong penetrating powers. Moreover, it was possible to obtain the complete disinfection in an exceedingly short time. Starting with the cold apparatus, and counting the time from the moment the furnace was lighted, complete disinfection could be obtained in 30 to 35 minutes.

I should add, to show the practicability of disinfection by simple current-steam machines, that blankets and articles of clothing which have been left in the steriliser for that time can be dried very rapidly by removing them from the steam chamber immediately after the completion of the sterilisation and exposing them freely to a current of air whilst they are still very hot. Owing to the bad conducting properties of most fabrics, more especially woollen ones, they retain heat long enough to cause a nearly complete evaporation of the water, which condenses on their surface when they are exposed to cold air. This process of drying is more specially applicable to clothes, blankets, &c., which can be rapidly spread out and form thin layers. Mattresses cannot be rapidly dried by this simple process.

In conclusion, I wish it to be understood that my object in writing these lines has not been to discuss the subject of disinfection by current steam in a complete manner, nor to give an account of my work in a scientific form. All I wished was to show how, by simple experiments, one can convince oneself of the efficiency of the simplest form of steam disinfection. Had I attempted to do more it would have been my duty to refer, at some length, to the work of Pasteur, Lister, Koch, Wolfhügel, Gaffky, Löffler, Esmarch, Klein, Parsons, Thresh, and many others.

LONDON:
Printed by JAS. TRUSCOTT & SON,
Suffolk Lane, E.C.

CHART illustrating results of the Bacteriological Survey of a single Water Supply.

A Soil immediately under turf covering unpolluted banks of an unpolluted feeder.
(F Soil polluted with cow dung; soil taken immediately under turf.)
B Unpolluted open feeders.
B′ Unpolluted land drain.
C Open feeders, with sources of pollution apparently distant from their banks.
D Open feeders and 1 well directly polluted.
E Polluting drain taking water mixed with fluid manure to a feeder.
F Manured soil.
G Reservoirs receiving few or slightly polluted feeders.
H Reservoirs receiving badly polluted feeders.
J Mains from slightly polluted reservoirs.
K Water taken from (1) Reservoir; (2) Main 1 mile from reservoir; (3) Same main 4 miles from reservoir.
L Shows changes in number of bacteria in water of unpolluted feeder receiving polluted water; bacterial contents of corresponding reservoir and main are also given.
M Special standards of purity for dry and wet weather obtained by the examination of a number of unpolluted feeders in this special water supply.

N.B.—The scale below 500 is 1 mm. to 10 bacteria.
above 1,000 is 14 mm. to 1,000.
above 35,000 is 20 mm. to 5,000.